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Serum macrophage-derived chemokine and interleukin 18 concentrations are associated with disease severity in children with atopic dermatitis.

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Summary

In the pathogenesis of AD, immunological disturbances play a central role. Immune dysregulation in AD includes increased production of inflammatory cytokines, elevated serum immunoglobulin E (IgE) levels and inflammatory cell infiltration. Defects in the innate and adaptive immune responses as well as T cell disturbances lead to and sustain allergic inflammation. Macrophage-derived chemokine (MDC/CCL22) and interleukin (IL)-18 are elements of both innate and adaptive immune responses whose role in the development of skin lesions is widely discussed. Serum MDC and IL-18 levels were assessed in 60 AD children (mean age 8.1 years) and 40 healthy controls (mean age 9.62 years) to investigate a possible associations between MDC and IL-18 concentrations and the clinical severity of the disease. The children were divided into two age groups (< 7 years old and ≥ 7 years old). We compared these groups for serum levels of MDC, IL-18, total IgE and blood eosinophils and analyzed their correlation with the severity of skin lesions, defined by the scoring atopic dermatitis (SCORAD) index.

The serum concentrations of MDC and IL-18 in AD patients were significantly higher than in healthy controls. The serum MDC levels significantly correlated with the SCORAD and other markers of allergic inflammation such as total IgE and eosinophil count in peripheral blood in both age groups. Serum IL-18 levels correlated positively with SCORAD index in the young children. In conclusion MDC and IL-18 serum concentrations may be useful inflammatory markers for assessing AD severity in children.

Key words

atopic dermatitis, cytokines, inflammation.

Atopic dermatitis (AD) is one of the most widespread, chronic inflammatory skin diseases characterized by distinctive morphology and localization of skin lesions, severe pruritus and presence of atopy in family history (1, 3, 25). AD affects at least 15% of children worldwide and can persist into adulthood. It is usually the first manifestation of allergic disease in children and can be the first step of the "atopic march" (1, 24, 31).

In the multifactorial pathogenesis of this disease, gene-environment interactions and immune system disturbances play a central role. Immune dysregulation in AD includes increased production of inflammatory cytokines, elevated serum immunoglobulin E (IgE) levels and inflammatory cell infiltration. Activation of T lymphocytes, dendritic cells, macrophages, keratinocytes, mast cells and eosinophils is characteristic of AD skin inflammatory response (1, 6, 25).

Stress, bacterial or viral infections, exposure to aero- or food-allergens as well as hygienic factors are considered to aggravate symptoms of AD (1). The pathogenesis of AD is mediated primarily by

CD4+ T lymphocytes that produce a type 2 helper T cell (Th2) cytokine profile, although recent studies indicate that both Th1 and Th2 cytokines may contribute to the lesions in AD. Increased expression of Th2 cytokines is observed in acute AD lesions while chronic lesions are associated with increased numbers of interleukin (IL)-5, IL-12, and interferon γ (IFN-γ) expressing cells (4, 14, 20, 23).

A crucial role in regulating the balance between Th1 and Th2 cytokines is played by IL-18 (23, 26).

IL-18 is a proinflammatory cytokine also known as INF-γ inducing factor. Recent studies revealed that IL-18 is a pleiotropic cytokine which plays a crucial role in both Th1 and Th2 lymphocyte-mediated immunity. It is produced by keratinocytes, monocytes, macrophages, dendritic cells (DCs) and osteoblasts and can induce the production of IgE and Th2 cytokines (1, 5). On the other hand, in the presence of IL-12 it can suppress the synthesis of IgE and demonstrates anti-allergic properties.

Additionally, IL-18 is an important element associated with the pathogenesis of AD in both innate and acquired immune system responses (30). IL-18 binds with Toll-like receptor and it is one of the first elements in the defence against the infection.

It has been reported that IL-18 can induce AD lesions independently of Th2 and IgE responses in mice. Moreover, in the presence of IL-3, IL-18 can directly stimulate basophils and mast cells to produce their mediators in IgE-independent manner (17). Elevated levels of IL-18 were observed in the stratum corneum of patients with AD, which reflects its production by keratinocytes (13).

Recent studies suggest that innate and adaptive immune responses are involved in the pathogenesis of AD (27). The increased expression of C-C chemokines as monocyte chemoattractant protein 4, eotaxin, macrophage-derived chemokine (MDC/CCL22), thymus and activation-regulated cytokine (TACK/CCL17), as well as cutaneous T cell–attracting chemokine (CTACK/CCL27) contributes to the infiltration of macrophages, eosinophils, and T cells into acute and chronic AD skin lesions (21, 22).

MDC, which is a member of the C-C chemokine family constitutively expressed by macrophages, monocytes and DCs, is one of the ligands for CCR4 (CC-chemokine receptor 4). It acts as a chemoattractant for CCR4-expressing cells such as memory T cells. MDC can induce selective recruitment of CD4+CD45RO+ T cells expressing Th2-type cytokines to the lesions in Th2-related diseases such as AD. It is also a chemoattractant for natural killer (NK) cells and eosinophils (8, 12).

Previous studies revealed that MDC production by activated T cells and monocytes is stimulated by Th2 cytokines such as IL-4 and IL-13, whereas it is downregulated by Th1 type cytokines such as IFN-γ (28). These data support the hypothesis that MDC expression is preferentially involved in Th2-type reactions. Recent studies have shown that serum levels of MDC are increased and correlate with disease activity in patients with AD [10].

AD is characterized by the complex interactions between the components of innate and adaptive immune system response. Despite extensive research efforts clinicians still do not possess a dependable laboratory marker for evaluation of AD severity in the pediatric population. Furthermore, there are no reliable biomarkers to monitor children with AD. IL-18 and MDC are elements of both adaptive and native immune systems and their serum concentrations, along with their relationship with other biomarkers of allergic inflammation, may potentially offer a new diagnostic tool.

Materials and methods

The present study included 60 children with AD (mean age 8.1 ± 4.45, range 2-17 yr) and 40 healthy controls (mean age 9.62 ± 4.94, range 2-17 yr) with no history of allergic diseases. Some changes in immunologic parameters are associated with age, therefore children from AD group and control group were divided into two age categories: younger (age range 2-6 yr) and older (age range 7-17 yr). AD was clinically diagnosed according to the criteria of Hanifin and Rajka (9),

TABLE 1. Demographic and clinical characteristics of AD children and healthy controls.

Clinical and laboratory findings	*AD group (n=60)*	*Control group (n = 40)*
Age (yr)	8.1 ± 4.45	9.62 ± 4.94
Sex (M/F)	24/36	17/23
SCORAD scores	31.1 ± 17.1	
< 15	14 (23%)	
15-40	30 (50%)	
> 40	16 (27%)	
Eosinophils (count/μl)	412.33 ± 274.25	185.22 ± 122.6
Serum total IgE (IU/ml)	680.52 ± 817.7	76.4 ± 125.02
Values presented as mean ± DS		

The clinical severity of the disease was estimated by the same investigator using the SCORAD (SCORing Atopic Dermatitis) index system ranging from 0 to 103 points (7). Antihistamines, topical steroids or calcineurin inhibitors were discontinued at least 1 week before enrollment into the study. The demographic and clinical characteristics of the patients are presented in Table 1. All of the experiments were approved by the Local Ethics Committee (Medical University of Silesia). Written informed consent was obtained from parents of all patients.

Measurements of IL-18 and CCL22 serum concentrations: blood was obtained in the morning, in a fasting state. Serum samples were obtained from clotted blood following centrifugation at 1300g at 4ºC for 10 minutes and were stored at -30ºC until analysis. Serum concentrations of IL-18 and MDC were measured with ELISA assay (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. The lower limits of detection were 12.5 pg /ml for IL-8 and 62.5 pg/ml for MDC.

Other laboratory investigations: serum concentration of total IgE was measured by ELISA using a commercial kit (Elecsys IgE II) according to the manufacturer's instructions. The lower limit of detection was 0,1IU/ml. Blood eosinophil count was determined using an automatic hematology analyzer.

Statistical analysis: the statistical analysis was performed using software package STATISTICA (Version 8.0). Data were analyzed using the Kruskall-Wallis and Mann-Whitney U-test. Correlation coefficients were determined using the Spearman rank correlation test. A P-value of < 0.05 was considered as statistically significant.

Results

Biomarkers of AD activity. The mean serum total IgE level of the AD children was significantly higher than that of the healthy children (680.52 ± 817.7 IU/ml vs 76.4 ± 125.02 IU/ml, $p<0.001$). The eosinophil count was significantly higher in AD patients than in the healthy controls (412.33 ± 274.25 counts/μl vs 185.22 ± 122.6 counts/μl, $p < 0.001$).

Serum IL-18 levels and AD severity. The mean serum concentration of IL-18 was significantly higher in the AD group compared to the healthy controls (89.62 ± 37.65 vs 57.29 ± 21.06 pg/mL, $p < 0.001$). Significant differences were observed in both younger (97.47 ± 39.81 vs 58.15 ± 18.61 pg/mL, $p < 0.001$) and older children (82.76 ± 34.83 vs 56.71 ± 34.83 pg/mL, $p = 0.002$). Positive correlation between serum IL-18 concentration and SCORAD index in AD children ($r = 0.36$, $p < 0.005$) was observed (Fig 1a).

This marker also showed a significant correlation with SCORAD index in young children ($r = 0.49$, $p = 0.006$). There was no correlation (Table 2, 3) between serum concentration of IL-18 and serum total IgE levels in either younger ($r = 0.22$, $p = 0.27$) or older children ($r = 0.13$, $p = 0.48$).

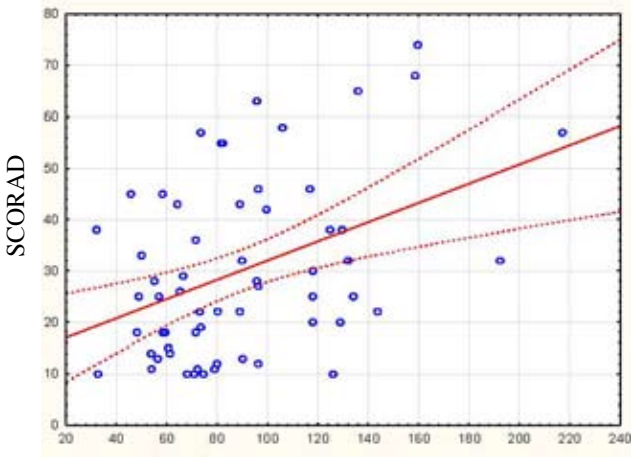

Fig. 1a: Positive correlation between serum concentration of IL-18 and SCORAD index in children with AD ($r = 0.36$, $p < 0.005$).

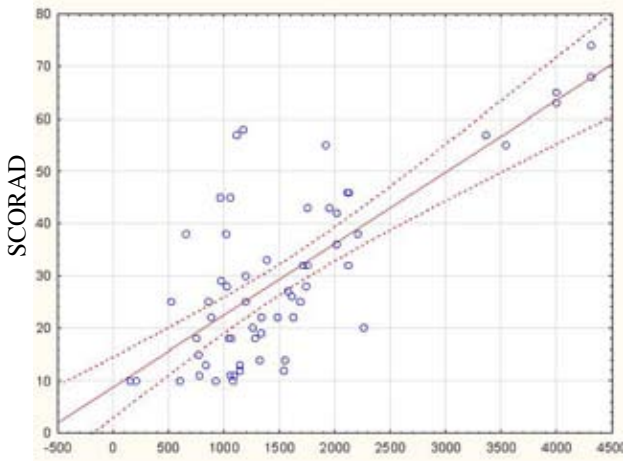

Fig. 1b: Positive correlation between serum concentration of MDC and SCORAD index in children with AD ($r = 0.62$, $p < 0.001$).

Serum MDC levels and AD severity. The mean serum concentration of MDC was significantly higher in the AD group compared to the healthy controls (1560.09 ± 962.98 vs 751.83 ± 209.43 pg/mL, $p < 0.001$).

Significant differences were observed in both younger (1818.14 ± 1074.74 vs 733.38 ± 217.22 pg/mL, $p < 0.001$) and older children (1334.3 ± 733.14 vs 764.12 ± 207.87 pg/mL, $p < 0.001$) of the study.

Positive correlation between serum MDC concentration (Fig 1b) and SCORAD index in AD children was observed ($r = 0.62$, $p < 0.001$). Serum MDC level significantly correlated with SCORAD score ($r = 0.81$, $p < 0.001$) and serum total IgE ($r = 0.53$, $p < 0.004$) in younger chil-

TABLE 2. Correlation between measured indices in younger children (Spearman's rank correlation).

	IL-18	*Total IgE*	*Eosinophil count*	*SCORAD*
MDC	0.51*	0.53*	0.28	0.81*
IL-18	-	0.22	0.39	0.49*
Total IgE	-	-	-	0.63*
* Indicates statistical significance with $p < 0.05$				

TABLE 3. Correlation between measured indices in older children (Spearman's rank correlation).

	IL-18	*Total IgE*	*Eosinophil count*	*SCORAD*
MDC	0.52*	0.22	0.56*	0.45*
IL-18	-	0.13	0.29	0.26
Total IgE	-	-	-	0.35
* Indicates statistical significance with $p < 0.05$				

dren. Moreover, in the older AD children group a significant correlation (Table 2, 3) between MDC and SCORAD index was observed ($r = 0.57$, $p = 0.001$).

IL-18 and MDC concentrations in patients with normal vs elevated IgE. In the AD group sixteen children had normal total IgE levels (41.3 ± 33.54 IU/ml), while others demonstrated elevated total IgE (912.96 ± 842.44 IU/ml). There was no significant difference in serum concentrations of IL-18 (AD with >IgE: 92.83 ± 39.89 pg/ml vs AD with normal IgE: 80.79 ± 30.02 pg/ml; $p > 0.28$) and MDC (AD with >IgE: 1675.14 ± 1024.91 pg/ml vs AD with normal IgE: 1243.68 ± 515.89 pg/ml; $p > 0.12$) in children with elevated vs normal total IgE concentrations.

AD children with elevated IgE also had a higher SCORAD index (AD with >IgE: 34.18 ± 17.66 vs AD with normal IgE: 18.75 ± 8.19; $p < 0.002$).

Discussion

The aim of our study was to investigate a possible associations between serum MDC and IL-18 concentrations and the clinical severity of the disease in AD children. We have confirmed that serum MDC and IL-18 concentrations are significantly higher in patients with AD and correlate with the severity of the disease.

Previous studies have demonstrated that the development of acquired immunity during childhood is associated with changes in immunologic parameters and age matching is important in evaluating the cytokine profiles of T cells (16). In the present study we have divided our patients into two age categories. We have confirmed that serum concentrations of IL-18 and MDC in children with AD are significantly elevated compared to those of healthy controls in both younger and older children.

Moreover, serum level of MDC correlated positively with SCORAD index in both age groups while IL-18 level correlated with SCORAD index only in the younger population.

We have also found a positive correlation between serum MDC levels and total serum IgE in the younger age group with AD. This finding suggests that MDC can be a useful marker in evaluating the disease activity in that age group of patients.

Significantly elevated serum concentrations of MDC were previously reported in adult patients with AD (10, 23). Other Authors observed a positive correlation between serum MDC levels, SCORAD index and eosinophils count in adult AD patients (15). Recent studies have shown a positive correlation between MDC concentrations and severity of AD in young children (19, 22).

The imbalance in chemokine serum concentrations plays a crucial role in the pathogenesis of AD (23). The production of Th2 mediated cytokines along with increased IgE levels and eosinophilia is well-known in the pathogenesis of AD. In the acute phase of the disease MDC induces a selective migration of Th2 lymphocytes to the allergic inflammation sites.

Although AD has been considered as an allergic Th2-mediated disease, recent studies have demonstrated a biphasic immunologic response with a switch from a Th2 to a Th1 phenotype in the chronic phase of the disease (1, 25).

One of the factors which plays a role in the maintenance of chronic AD lesions and production of Th1 cytokines is IL-18 [26]. IL-18 is a pleiotropic proinflammatory cytokine that is a component of both innate and adaptive immune systems. Recent studies have indicated that serum IL-18 concentration may be associated with AD severity (13, 17, 18).

In the present study serum IL-18 concentration was elevated in both the younger and the older children with AD. IL-18 levels correlated positively with the disease severity measured by SCORAD index in the younger age group.

We have not found a statistically significant correlation between IL-18 and total IgE or eosinophil counts in either age group of patients, or between IL-18 and SCORAD index in the older children.

In previous studies it was reported that increased IL-18 concentration was positively correlated with total IgE and severity of the disease in older children and adult patients with AD (18). Hon et Al. observed a significant positive correlation between serum IL-18 levels and SCORAD index in young children (11). Other authors reported

that serum concentration of IL-18 in young children with AD was significantly lower than that of healthy controls (23). Elevated levels of IL-18 in school children with AD, which correlated with severity of the disease and total serum IgE, has been observed previously (2, 18). However, we did not confirm that observation in our study.

Recent data showed that IL-18 may be responsible for inducing skin lesions such as those present in AD independently of IgE elevation (17). We have not observed significant differences in serum IL-18 concentrations in AD children with elevated versus normal total IgE. In both groups serum IL-18 concentrations were significantly higher than those of healthy controls; however, children with elevated total IgE had higher SCORAD index values. This suggests that IL-18 may be responsible for AD development in patients with intrinsic AD. Unfortunately, this group was relatively small in our study and it needs further investigation.

In conclusion, in our study we have analyzed elements of both Th1 and Th2 immune response. Elevated levels of IL-18 and MDC confirm their involvement in the pathogenesis of AD in children and indicate that they can be useful markers of disease severity. In our study the cytokines showed much stronger correlation with SCORAD index in the young children which suggests that MDC and IL-18 are better inflammatory markers in assessing AD in that age group.

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References

1) Akdis C.A., Akdis M., Bieber T. et Al. - Diagnosis et treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/ PRACTALL Consensus Report. Allergy 61(8), 969-987, 2006.
2) Aral M., Arican O., Gul M. et Al. - The relationship between serum levels of total IgE, IL-18, IL-12, IFN-γ and disease severity in children with atopic dermatitis. Mediators Inflamm 2006 (4): 73098.
3) Bieber T. - Atopic Dermatitis. Ann. Dermatol. 22, 125-137, 2010.
4) Boguniewicz M., Leung D.Y. - Atopic dermatitis: a disease of altered skin barrier and immune dysregulation. Immunological Reviews 242, 233-246, 2011.
5) Dinarello C.A. - Interleukin 1 and interleukin 18 as mediators of inflammation and the aging process. Am. J. Clin. Nutr. 2, 447-455, 2006.
6) Eichenfield L.F., Ellis C.N., Mancini A.J. et Al. - Atopic dermatitis: epidemiology and pathogenesis update. Semin Cutan Med Surg 31 (suppl), S3-S5, 2012.
7) European Task Force on Atopic Dermatitis. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. Dermatology 186 (1), 23-31, 1993.
8) Godiska R., Chantry D., Raport C.J. et Al. - Human macrophage-derived chemokine (MDC), a novel chemoattractant for monocytes, monocyte-derived dendritic cells, and natural killer cells. J. Exp. Med. 185, 1595-1604, 1997.

9) Hanifin J.M., Rajka G. - Diagnostic features of atopic eczema. Acta Dermatol Venerol 92, 44-47, 1980.
10) Hashimoto S., Nakamura K., Oyama N., et Al. - Macrophage-derived chemokine (MDC)/CCL22 produced by monocyte derived dendritic cells reflects disease activity in patients with atopic dermatitis. J. Dermatol. Sci. 44, 93-99, 2006.
11) Hon K.L., Leung T.F., Ma K.C. et Al. - Serum concentration of IL-18 correlates with disease extent in young children with atopic dermatitis. Pediatr. Dermatol. 21, 619-622, 2004.
12) Imai T., Nagira S., Kakizaki M. et Al. - Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. Int. Immunol. 11, 81-88, 1999.
13) Inoue Y., Aihara M., Kirino M. et Al. - Interleukin-18 is elevated in horny layer in patients with atopic dermatitis and is associated with atopic dermatitis colonization. Br. J. Dermatol. 164, 560-567, 2011.
14) Kabashima K. - New concept of the pathogenesis of atopic dermatitis: Interplay among the barrier, allergy, and pruritus as a trinity. J. Dermatol. Sci. 70, 3-11, 2013.
15) Kakinuma T., Nakamura K., Wakugawa M. et Al. - Serum macrophage-derived chemokine (MDC) levels are closely related with the disease activity of atopic dermatitis. Clin. Exp. Immunol. 127, 270-273, 2002.
16) Kawamoto N., Kaneko H., Takemura M. et Al. - Age-related changes in intracellural cytokine profiles and Th2 dominance in allergic children. Pediatr. Allergy Immunol. 17, 125-133, 2006.
17) Konishi H., Tsutsui H., Murakami T. et Al. - IL-18 contributes to the spontaneous development of atopic dermatitis-like inflammatory skin lesion independently of IgE/stat6 under specific pathogen free conditions. PNAS 17, 11340-11345, 2002.
18) Kou K., Aihara M., Matsunaga T. - Association of serum interleukin-18 and other biomarkers with disease severity in adults with atopic dermatitis. Arch. Dermatol. Res. 304, 305-312, 2012.
19) Leung T.F., Ma K.C., Hon K.L. et Al. - Serum concentration of macrophage-derived chemokine may be a useful inflammatory marker for assessing severity of atopic dermatitis in infants and young children. Pediatr. Allergy Immunol. 14, 296-301, 2003.
20) Machura E., Mazur B., Golemiec E. et Al. - Staphylococcus aureus skin colonization in atopic dermatitis children is associated with decreased INF-gamma production by peripheral blood CD4+ and CD8+ Tcells. Pediatr. Allergy Immunol. 19, 27-45, 2008.
21) Machura E., Rusek-Zychma M., Jachimowicz M. et Al. - Serum TARC and CTACK concentrations in children with atopic dermatitis, allergic asthma and urticaria. Pediatr. Allergy Immunol. 23, 278-284, 2012.
22) Nakazato J., Kishida M., Kuroiwa R., et Al. - Serum levels of Th2 chemokines, CCL17, CCL22, and CCL27, were the important markers of severity in infantile atopic dermatitis. Pediatr. Allergy Immunol. 19, 605-613, 2008.
23) Narbutt J., Lesiak A., Sysa-Jędrzejowska A. et Al. - The imbalance in serum concentration of Th-1- and Th-2- derived chemokines as one of the factors involved in pathogenesis of atopic dermatitis. Mediators of Inflammation 2009; doi:10.1155/2009/269541.
24) Odhiambo J.A., Williams H.C., Clayton T.O. et Al. - Global variations in prevalence of eczema symptoms in children from ISAAC Phase Three. J. Allergy Clin. Immunol. 124, 1251-1258, 2009.
25) Schneider L., Tilles S., Lio P. et Al. - Atopic dermatitis: a practice parameter update 2012. J. Allergy Clin. Immunol. 131, 295-299, 2013.
26) Trzeciak M., Sokołowska-Wojdyło M., Barańska-Rybak W. et Al. - Interleukin 18- a pleiotropic cytokine involved in the Th1 and Th2 immunological response. Post Dermatol Allergol. XXVIII, 4, 309-312, 2011.
27) Vulcano M., Albanesi C., Stoppacciaro A. et Al. - Dendritic cells as a major source of macrophage-derived chemokine/CCL22 in vitro and in vivo. Eur. J. Immunol. 31, 812-822, 2001.
28) Wollenberg A., Klein E. - Current aspects of innate and adaptive immunity in atopic dermatitis. Clinic Rev. Allerg. Immunol. 33, 35-44, 2007.
29) Yamashita U., Kuroda E. - Regulation of macrophage-derived chemokine (MDC, CCL22) production. Crit. Rev. Immunol. 22, 105-114, 2002.
30) Yoshimoto T., Tsutsui H., Tominaga K. - IL-18, although antiallergic when administered with IL-12, stimulates IL-4 and histamine release by basophils. Proc. Natl. Acad. Sci. USA 96, 13962-13966, 1999.
31) Zheng T., Yu J., Oh M.H., Zhu Z. - The Atopic march: progression from atopic dermatitis to allergic rhinitis and asthma. Allergy Asthma Immunol. Res. 3, 67-73, 2011.